

REPORT DOCUMENTATION PAGE					Form Approved OMB No. 0704-0188	
<p>The public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing the burden, to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.</p> <p><b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b></p>						
1. REPORT DATE (DD-MM-YYYY) 27/5/08		2. REPORT TYPE Final Technical Report			3. DATES COVERED (From - To) 11.14.05-5.14.08	
4. TITLE AND SUBTITLE Postdoctoral Fellowship for Dr. Lindholm, Underwater Physiology and Medicine				5a. CONTRACT NUMBER GRANT #: N00014-05-1-0076		
				5b. GRANT NUMBER GRANT #: N00014-05-1-0076		
				5c. PROGRAM ELEMENT NUMBER GRANT #: N00014-05-1-0076		
				5d. PROJECT NUMBER GRANT #: N00014-05-1-0076		
6. AUTHOR(S) Claes E.G. Lundgren (clundgre@buffalo.edu) and David R. Pendergast, Ed.D. (dpenderg@buffalo.edu)				5e. TASK NUMBER GRANT #: N00014-05-1-0076		
				5f. WORK UNIT NUMBER GRANT #: N00014-05-1-0076		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Center for Research and Education in Special Environments, Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences at the University at Buffalo, Buffalo, NY 14214					8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Research Foundation State of New York, University at Buffalo Charles J Kaars Assistant Vice President, Sponsored Projects Services 402 Crofts Hall University at Buffalo, Buffalo, NY					10. SPONSOR/MONITOR'S ACRONYM(S) ONR	
					11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Distribution Unlimited						
20080603194						
13. SUPPLEMENTARY NOTES none						
14. ABSTRACT <p>The objective of this Postdoctoral fellowship was to provide education to extend their training to embarking on an independent research. Two Postdoctoral Fellows were trained. Important safety issues of breath-holding and limitations to diver performance were studied. Two studies showed the potential for increased risk of loss of consciousness (LOC) during breath-holding when carbohydrate stores were reduced by either dietary restriction or exercise without proper replenishment of glucose and glycogen. In addition, it was shown that the risk of LOS is grater in experienced breath-hold divers due to increased CO2 tolerance. The increased risk of LOC could be prevented by a high carbohydrate drink prior to diving. In another series of studies it was shown that there are respiratory limitations to sustained exercise performance in divers in air and during surface and at depth (120 fsw) swimming, and that these limitations could be eliminated by specific training of the respiratory muscles (RMT). RMT minimized respiratory muscle fatigue and normalized the ventilatory response to increasing CO2 (CO2 sensitivity) and blood CO2 in CO2 retaining divers which would reduce the potential of CO2 poisoning and oxygen breathing effects.</p>						
15. SUBJECT TERMS Postdoctoral Fellow, university research, breath-hold diving, diver exercise performance, respiratory muscles, swimming, pulmonary function						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 25	19a. NAME OF RESPONSIBLE PERSON David R. Pendergast	
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (Include area code) 716-829-3830	

## **FINAL TECHNICAL REPORT**

**GRANT #:** N00014-05-1-0076

**PRINCIPAL INVESTIGATOR:** Claes E.G. Lundgren ([clundgre@buffalo.edu](mailto:clundgre@buffalo.edu)) and David R. Pendergast, Ed.D. ([dpenderg@buffalo.edu](mailto:dpenderg@buffalo.edu))

**INSTITUTION:** Center for Research and Education in Special Environments, Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences at the University at Buffalo, Buffalo, NY 14214

**GRANT TITLE:** Postdoctoral Fellowship for Dr. Lindholm, Underwater Physiology and Medicine

**AWARD PERIOD:** 11.14.05 – 5.14.08

### **OBJECTIVE:**

To provide an environment and education for Dr. Peter Lindholm, M.D. and Dr. Andrew Ray, PhD, that will allow them to extend their current medical and research training to embarking on an independent research and teaching career. This will include participation in ongoing funded research and the development of new research projects based on their current research interests, as well as new ones, and writing research grant proposals. In addition, providing familiarization with the academic administrative and teaching environments of a research university. Training will also involve training in directing graduate students and research personnel, participation in group discussions, scientific writing and delivering and attending seminars, as well as participation in national scientific meetings.

The major aim of this effort is to provide an environment and funding for Dr. Lindholm and Ray to develop into independent scientists and teachers with interests in applied, environmental and undersea research, teaching and medicine. This will be accomplished by their participation in these activities in CRESE and the Department of Physiology. Initially Dr. Lindholm and Ray will be immersed in ongoing research to gain an understanding of the operation of projects, specifically supervising students and technicians, recruiting subjects, collecting and analyzing data and writing papers and reports, as well as the administrative responsibilities of research. Dr. Lindholm and Ray will be gradually introduced to teaching responsibilities of a "tenure track" faculty member, initially in an undergraduate course and later on in the Medical and Graduate courses.

As part of this experience Dr. Lindholm participated in two experimental projects. The first project was a continuation of Dr. Lindholm's previous work on breath-holding. Breath-holding is an integral part of Navy divers training and operational requirements. Clearly, divers have to be able to perform breath-holds safely, and currently there are no fool proof standards for either training or performance. It is known that hyperventilation prior to breath-holding reduces  $P_{CO_2}$ , prolongs breath holding times, and may result in  $P_{O_2}$  dropping below critical levels. In fact several accidents occur annually during breath-hold diving, and deaths among Navy divers have happened during breath-hold dive training (information from Navy representative at SOCOM).

The second project for Dr. Lindholm was to test the hypothesis that respiratory muscle training will enhance the swimming performance of divers by increasing the efficiency of the respiratory muscles, thus allowing the muscles involved in swimming to not be compromised. We propose that this would result from two interrelated factors, first the total ventilation would be reduced, and be associated with an increased tidal volume and reduced breathing frequency, thus increased ventilatory efficiency. Secondly, due to the increased respiratory muscle efficiency and resultant sustained locomotor muscle capacity, less lactic acid would be produced by the former muscles, requiring less respiratory compensation for metabolic acidosis. The reduced total ventilation would not only improve swimming endurance, but also enhance the safety of the divers by minimizing the stress of hyperventilation that can cause panic and accidents. These experiments were conducted during swimming at the surface and at 4 fsw.

The line of investigation pursued by Dr. Lindholm was continued by Dr. Ray. Namely the investigation of the effects of respiratory muscle training on pulmonary and exercise performance in divers at depths of 55 and 120 fsw. In addition, Dr. Ray investigated the quantification of the work of breathing at these depths, and the effect of respiratory muscle training.

**APPROACH:** This program to developing young scientists, first recruited a promising recent MD graduate, Dr. Peter Lindholm, with interests in applied systems integrated physiology with emphasis on environmental and undersea interests and developed his independence as an investigator. The second step was to integrate him into an existing research program to support him while he gained experience in the process of research. During this phase he began thinking about extensions of his current work on breath-hold diving to new areas where he could collect data and publish papers on which his future independent funding will be based. Finally he developed his own independent research projects and will apply for funding. During the second phase of his program teaching was introduced in the Medical and Graduate Schools. After one year Dr. Lindholm complete the first phase of his program, and was successful in gaining a position at the Karolinska Hospital and he returned to Sweden. During this transition, we recruited Dr. Andrew Ray to fill the position and he underwent the same training program as outlined above for Dr. Lindholm. After two and one-half years, Dr. Ray has succeeded in gaining a faculty position in the Department of Rehabilitation Sciences at the University at Buffalo, Buffalo, NY.

#### **ACCOMPLISHMENTS:**

There are two major accomplishments of this postdoctoral award, first is that two professionals were trained to pursue research careers, as demonstrated by the fact that they both have received University research positions. The second is that significant contributions to two research areas has been accomplished, i.e. breath-hold diving and the effect of respiratory muscle training on performance in divers. In addition, in conjunction with the training of Dr. Lindholm and Ray two graduate students (M. Conniff and A. Simpson) completed their MS and several undergraduate students had the opportunity to participate in these studies. The studies sponsored by this grant resulted in the publication of 5 papers published in reviewed journals and one submitted to a reviewed journal and 8 abstracts and presentations at national meetings. In addition the work completed under this grant was presented at the Navy Progress Review Meeting from 2006-2008.

Dr. Lindholm's first project was an extension of his previous work on the relationship between breath-holding time and the substrate used during the breath-hold. To avoid drowning, a breath-hold (BH) diver has to surface in time to start breathing before losing consciousness due to hypoxia. Normally, a strong urge to breathe forces the diver to end the breath-hold dive while the falling alveolar oxygen level is still within safe limits. The urge to breathe is mainly driven by the rising level of  $\text{CO}_2$  in the blood that is produced by metabolic processes, but a hypoxic ventilatory drive is also involved in dry BH or shallow breath-hold dives. The interaction between the hypercapnic and hypoxic ventilatory drives has been shown to influence the apnea breaking point. We previously showed that during a state of increased lipid metabolism, imposed by 18 hrs of carbohydrate abstinence and 2 hrs of aerobic exercise, breath-holding subjects terminated their breath holds at lower end-tidal oxygen and  $\text{CO}_2$  pressures than during control conditions. The reduced rate of  $\text{CO}_2$  production during primarily lipid metabolism caused the subjects to rely more on their hypoxic ventilatory drive, as is the case when  $\text{CO}_2$  stores have been lowered by hyperventilation prior to apnea. The continued elevation in metabolism post-exercise may have contributed to increased  $\text{O}_2$  consumption during the breath-holds and may also have increased lipid metabolism as reflected by the lowered respiratory exchange ratio (RER). The combination of these factors results in increased risk of hypoxic loss of consciousness.

A short period of fasting reduces blood glucose and RER. A reduction in carbohydrate metabolism with a concomitant increase in lipolysis results in lower  $\text{CO}_2$  production for a given energy production, a situation that could delay the  $\text{CO}_2$  build-up and the urge to breathe, thus making a longer breath-hold possible. The increased breath-hold duration would increase  $\text{VO}_2$  and result in lower arterial  $\text{PO}_2$  at the end of the breath-hold which would decrease the safety of the dive. We hypothesized that an 18 h fast (compared to an 18h fast with subsequent carbohydrate ingestion) would yield lower blood glucose, increase lipid metabolism and reduce carbohydrate metabolism, and that breath-holds following fasting would be of longer duration, and that both the time to reach the physiological breaking point (PBP) and the maximal break-point (MBP) would be delayed. It was further hypothesized that, when subjects replenished their blood sugar levels with carbohydrate containing food and drinks, the breath-hold durations (PBP and MBP) would be shorter and breath-holds would be terminated with higher, and safer, end-tidal  $\text{PO}_2$  and arterial oxygen saturation ( $\text{SaO}_2$ ) compared to breath-holds in the fasting condition.

The principal finding of the present study was that breath-hold duration was increased by fasting and decreased by administration of carbohydrate rich food and drink after fasting. Also, during fasting, breath holding resulted not only in longer breath-hold durations, but in lower  $\text{PETO}_2$  and  $\text{PETCO}_2$  at the physiological breakpoint, compared to breath-holding after carbohydrate feeding. This suggests that breath holding after fasting may entail an increased risk of serious hypoxia.

When the human body utilizes lipids instead of carbohydrates as an energy source, the ratio between the amount of carbon dioxide produced and the amount of oxygen consumed decreases by almost 30%. Since the arterial level of  $\text{CO}_2$  constitutes the primary respiratory drive, predominant lipid metabolism should theoretically prolong the time until an irresistible  $\text{CO}_2$  dependent respiratory drive is reached during apnea. Conversely, food intake by subjects who have fasted for more than 12 hrs has been shown to increase blood glucose, increase RER and induce thermogenesis (heightening energy consumption).

The effect fasting had on post-apneic pulmonary gas concentrations in the present study was less than we previously reported, for subjects that had depleted their carbohydrate stores using a combination of carbohydrate free diet and long duration aerobic exercise. In that study breath holds after the diet and exercise regime showed significantly lower  $\text{CO}_2$  and  $\text{O}_2$  pressures at maximum breath hold breaking points, but no effect on breath hold duration was found. There are probably two reasons for the seemingly different results of the two studies. The first is that due to fasting for 18h liver and blood glucose probably were compromised, while after carbohydrate free diet combined with exercise both blood and liver glucose as well as muscle glycogen were also likely to be compromised. The second is that the subjects in the present study were not trained breath holders, whereas in the first study all subjects were experienced breath hold divers. Trained breath hold divers were able to withstand more than twice the respiratory drive compared to the subjects in the present study.

It is well known that the respiratory drive is dependent on the interaction between hypercapnic and hypoxic stimuli. During normo- or hypocapnia the respiratory drive is not affected by hypoxia until  $\text{PaO}_2$  is down to 6-7 kPa. However, during hypercapnia the respiratory drive is increased by less severe hypoxia. During breath-holding, the carbon dioxide sensitivity is slightly increased as soon as the alveolar oxygen level is decreased below normoxia, but the relationship between alveolar oxygen and alveolar carbon dioxide at breath-hold breaking point becomes appreciably steeper first when the oxygen pressures declines below 7.5-7 kPa. The average end tidal oxygen concentrations at breaking point of maximum duration breath-holds are below 7 kPa for the experienced breath-hold subjects; whereas for the subjects in the present study break point oxygen levels, although depressed, still remained above 7 kPa. We postulate that the experienced breath hold subjects depended to a large extent on their hypoxic drive, whereas the less experienced subjects in the present study aborted their breath holds at or slightly before the hypoxia started to enhance the hypercapnic respiratory drive. Thus, although there was no fixed  $\text{PCO}_2$  at termination in the present study, the  $\text{PCO}_2$  in all conditions were close to 6 kPa. In the previous study, the relationship between  $\text{PO}_2$  and  $\text{PCO}_2$  at break point was much steeper (Fig 4), i.e. even slight changes in generally low oxygen levels markedly affected the carbon dioxide pressures experienced divers were able to breath hold against, whereas the inexperienced breath-holders showed higher and relatively stable sensitivity to carbon dioxide over a range of higher oxygen levels.

Another aspect of Dr. Lindholm's development was to examine the physiology of competitive static breath hold competitors. It is known that hyperventilation reduces the urge to breathe from hypercapnia during a breath-hold (BH), sometimes causing motivated individuals to hold the breath until loss of consciousness (LOC), BH diving instructors generally discourage hyperventilation. Even though the hypoxic ventilatory drive is also involved in the urge to breathe, and will stimulate breath-hold divers to resume breathing, this stimulus has been considered too weak to ensure safe breath-hold diving. Every year recreational swimmers unaware of the dangers of hyperventilation drown. Nonetheless, athletes competing for duration in immersed breath-holding (Static Apnea) typically hyperventilate (personal observation) before performances, yet only about 10% surface with symptoms of severe hypoxia such as loss of motor control (LMC) or rarely loss of consciousness. While studies on acute hypoxia at altitude have been driven by its practical importance for aviation, little information is available on acute asphyxia in breath-hold diving. Therefore, this study was designed to explore the level of hypoxia commonly experienced during Static Apnea as related to the specific endpoints of LMC and LOC. We hypothesized that

hyperventilation, in preparation for prolonged breath-holding by trained BH-divers, would preclude hypercapnia to reach conventional breaking-point levels (in the absence of preparatory hyperventilation).

The reasoning for discouraging hyperventilation before underwater swimming seemingly does not strictly apply to Static Apnea. The obvious reason for this is that the swimming BH diver consumes oxygen at a much higher rate than the resting diver. Thus, in the swimmer, the time between hypoxic chemoreceptor stimulation forcing an ascent and arterial blood PO<sub>2</sub> is often short enough to cause LOC, while the Static Apnea diver who is resting has more time to react to clues for terminating the breath-hold. Furthermore, it seems as if the levels of tolerable hypoxia described for acute high-altitude exposures correspond fairly well to the tolerance to acute hypoxia due to apnea observed presently. This appears reasonable since in both cases hypocapnia or normocapnia is common.

Breath-hold divers in this study managed to surface and exhale into a sampling tube for end-tidal gas after about 5 min of breath-holding with end-tidal PO<sub>2</sub> levels as low as 20 mmHg, with LMC, and as low as 22-23 mmHg without LMC/LOC. The divers hyperventilated extensively so as to be hypocapnic before breath-holding and were still hypocapnic or normocapnic at the termination of breath-holding. If we consider LOC instead of the urge to breathe as the end-point of a breath-hold the effect of hyperventilation to increase safe BH-duration can be ascribed to an increase in oxygen stores which can be calculated to suffice for more than one minute of extra BH time before LOC. Moreover, trained breath-hold divers have been shown to have a gradually reduced oxygen uptake from the lungs during long breath-holds. Note that there is typically a very tight temporal coupling between LMC/LOC, and LOC is in other settings (e.g. altitude), typically expected at a PO<sub>2</sub> of about 30 mmHg which is well above our present observations. Hyperventilation for an extended duration may cause profound hypocapnia with associated neurological symptoms such as paresthesias and tetanus. The divers were all hypocapnic when starting the BH but, in contrast to what would be expected, neither did they report symptoms nor were any signs of hypocapnia observed. All divers were able to handle the sampling tube and exhale into it prior to breath-holding. An unanswered question is whether this lack of sensitivity to hypocapnia is due to adaptation similar to what is known to occur in mountaineers although in the latter category the hypoxia exposure is more sustained which probably is a condition for the changes in bicarbonate levels of the cerebrospinal fluid which account for the acclimatization. When the carbon dioxide content in the blood has been reduced by hyperventilation, the drive to breathe is abolished, thus making it possible to hold the breath for a period longer than the time it takes to reach critical hypoxemia. This is very dangerous and accidents (sometimes fatal) occur every year. Nonetheless, apnea athletes almost always hyperventilate before diving. They use a mode of ventilation that consists of slow and deep breaths; approx 4-6 breaths/minute (personal observation) for an extended period. Our subjects hyperventilated for approximately 6-8 minutes before each apnea including warmups, alternating deep slow breathing with faster deep breathing. Remarkably, these divers manage to sense when to surface to avoid LOC, a notion that suggests that they rely on the hypoxic ventilatory drive or other cues to know when to abort breath-holding. Some divers describe that they surface when their vision changes (grey-out) while others mention some other non-specific sensation of altered mental state. The most reliable data on the effects of acute hypoxia is available in the literature on aviation medicine. Macmillan reports a "time of useful consciousness" of 40 seconds after an explosive decompression to an altitude of 10,668 m (ambient pressure 179 mmHg)

while breathing air. Depending on  $\text{PACO}_2$  which may range between 20 and 40 mmHg,  $\text{PAO}_2$  will be 24-19 mmHg. The interaction between  $\text{PaO}_2$  and  $\text{PaCO}_2$  in influencing LOC has been described by Ernsting as follows: "Consciousness is lost when the jugular venous oxygen tension is reduced to 17-19 mmHg. The corresponding cerebral arterial oxygen tension varies with cerebral blood flow, which itself depends upon the arterial tensions of oxygen and carbon dioxide. Thus the arterial oxygen tension that produces a jugular venous tension sufficiently low to cause unconsciousness can lie between 20 and 35 mmHg depending on the degree of hypocapnia. Accordingly, although consciousness is usually lost when the alveolar oxygen tension is reduced to 30 mmHg or below for a significant period of time, it is possible to lose consciousness with an alveolar oxygen tension as high as 40 mmHg if there is marked hyperventilation, or to retain consciousness at an alveolar oxygen tension as low as 25 mmHg if there is no hypocapnia." However, during apnea the level of hypoxia will not be stable, and thus the exact duration of "useful consciousness" during apnea is difficult to predict. An important point made by Ernsting is that hypocapnia impedes oxygen transport to the brain at two levels; partly by reducing brain perfusion and partly by shifting the  $\text{O}_2$  dissociation curve of hemoglobin to the left.

There are some previous publications on ET  $\text{PO}_2$  in breath-holding: it has been reported that an exhaled  $\text{PO}_2$  of 29 and 28 mmHg after resting non-immersed apneas of 270 and 300 seconds, respectively. Hyperventilation followed by apnea during exercise has been reported to produce convulsions, ET  $\text{PO}_2$  was measured to values between 22 and 26 mmHg. The same author also did BH without prior hyperventilation (and thus higher end apnea  $\text{CO}_2$ ). These tests did not cause convulsions at ET  $\text{PO}_2$  of 26 mmHg. In the classic work showing the physiological basis of ascent blackout, Lanphier and Rahn describe one subject hyperventilating and then exercising lightly on an ergometer cycle during a simulated dive to 2 ATM (10m) in a pressure chamber: "upon ascent, his  $\text{PO}_2$  dropped to 24mmHg,  $\text{O}_2$  uptake ceased, and there is evidence that  $\text{O}_2$  was being extracted from the blood. Impairment of consciousness occurred." Field test on one diver during no-limit assisted diving has reported ET  $\text{PO}_2$  of 24 mmHg without LOC. However, the severity of hypoxia close to loss of consciousness during apnea seems to be of similar intensity as in the "steady state" situation of hypobaric hypoxia during breathing.

Dr. Lindholm continued his research development by participating in a study to confirm previous studies showing that respiratory muscle training improved exercise performance when performed 5 days per week by comparing that effect to training 3 days per week. In comparison to exercise on land, respiratory work during underwater exercise is increased due to the hydrostatic pressure differences across the chest as well as increased flow resistive respiratory work. Previous studies have demonstrated an increased work of breathing at rest and particularly during exercise while utilizing self-contained underwater breathing apparatus (scuba) at depth. The increased work of breathing is principally due to added airflow resistance from both the apparatus and increased gas density. This is likely to require augmented oxygen delivery via increased blood flow to respiratory muscles. It has recently been shown in healthy individuals that ventilatory limitations may cause a reduction of maximal exercise performance on land. The weakened exercise capacity has been attributed to a reduction in locomotor muscle oxygen transport secondary to diminished locomotor muscle blood flow. In several studies on land, respiratory muscle fatigue has been reported as a contributing factor to reduced maximal and endurance exercise performance. These same factors may also limit exercise performance in divers.

Leith and Bradley (1976) were the first to demonstrate that respiratory muscle strength and endurance can be improved through specific respiratory muscle training (RMT). More recently,

significant improvements in whole-body exercise endurance on land following specific respiratory muscle training have been documented in elite athletes. It has recently been shown in our laboratory that resistive RMT (RRMT) which is performed by vital capacity maneuvers against spring loaded breathing valves imposing about 50 cmH<sub>2</sub>O inspiratory and expiratory opening pressures (which is 25 -50% of max pressures) five days per week for four weeks significantly improved swimming endurance at the surface and at 1.22 m under water (referenced to the membrane of a back-mounted breathing regulator). These improvements were greater than those achieved by voluntary isocapnic hyperpnoea which has been employed in many other land based studies. Training five days per week may be too intense to maximize improvements in respiratory and fin swimming muscles. We reasoned that reducing the number of training days to three may result in greater improvements in swimming performance as has recently been shown for locomotor muscles. On the other hand, three days per week for 4 weeks of RRMT may not provide a sufficient stimulus for adaptation and thus performance improvement may not occur, or be less than that of RRMT-5.

The purpose of this study therefore, was to evaluate whether resistive respiratory muscle training three days per week for four weeks could improve respiratory function and surface swimming (with snorkel) and underwater swimming performance while utilizing scuba to the same degree as previously shown for a training schedule of five days per week for four weeks. In addition, we hypothesized that the improvements after RRMT-3 could be sustained over three months by RRMT twice per week (RRMT-M).

The present study demonstrated that both RRMT-3 and RRMT-5 significantly improved respiratory muscle strength and also improved respiratory endurance. Both RRMT-3 and RRMT-5 resulted in a decreased frequency, reduced ventilation and oxygen consumption and prolonged endurance swim times. Furthermore RRMT-M for three months maintained the improvements observed after RRMT-3. RRMT in this study primarily improved maximal inspiratory and expiratory pressures and respiratory endurance (RET). The improvements in pressures in both RRMT-3 and RRMT-5 were less than observed by Leith and Bradley (1976) as they used maximal resistance and this study only used  $\pm 50$  cm H<sub>2</sub>O (~40% of max). RRMT-3 and -5 significantly increased RET (73 and 217%, respectively), which is in agreement with previous studies in divers. There were no significant changes in pulmonary function after 3 or 5 days per week of RRMT which is agreement with the observations by Leith and Bradley (1976) and data from our laboratory. Studies that used voluntary isocapnic hyperpnoea showed increased pulmonary function and RET, but not altered  $P_{exp}$  and  $P_{insp}$ . Based on these data, improving  $P_{exp}$  and  $P_{insp}$  (15%) and RET (73%) is sufficient to result in improvements in respiratory performance, as well as fin swimming endurance. The demonstration in the present study of an ergogenic effect of RRMT is in agreement with a previous study of fin swimming and many studies of land exercise.

The underwater diving environment imposes increased respiratory loads on divers and thus challenges respiratory muscles. Respiratory muscle fatigue may result as a consequence of these challenges. The work of breathing increases as the density of the inhaled gases increases. Although our subjects were tested at a depth of only 1.22 m, limiting the increase in gas density to 13% (compared to surface conditions), it has been speculated that increases in lung volumes underwater would increase inspiratory flow rate, and force the inspiratory muscles to develop more

tension. This is so because at the larger lung volumes a stronger recoil pressure from the chest-lung combination has to be overcome for inspiration. This could contribute to increased respiratory muscle energy utilization and respiratory muscle fatigue during underwater swimming with scuba. In addition to the increased work of breathing associated with high gas density underwater, a reduction of the end-expiratory lung volume will shorten the resting length of the diaphragm and consequently lower the point on the length-tension curve from which it operates. Reducing the resting length of a canine diaphragm to 70% of the optimal length lowered the maximal force generation at a stimulation frequency of 100 Hz by 40%. As exercise intensity and gas density increase, the added resistive work of breathing leads to increases in both FRC and alveolar  $\text{CO}_2$  tension. If resting length limits the ability of the respiratory muscles to generate force, compensation can be made temporarily by increasing the firing frequency, which however renders the diaphragm much more susceptible to fatigue (29).

Considering the hyperventilation at the end of the endurance swim seen pre-RRMT in most subjects in this study, it is reasonable to speculate that this was due to stimulation from lactate originating in respiratory and/or locomotor muscles. This might have led to respiratory muscle fatigue, generating reduced tidal volumes and increased frequency and consequently increased energy cost of respiration, respiratory muscle blood flow, and reduced blood flow and oxygen delivery to other exercising muscles. This cascade of events is likely to have led to the termination of exercise pre-RRMT and it is noteworthy that there was no paradoxical terminal hyperpnoea post-RRMT whether the training had been conducted 3 or 5 days weekly.

Following RRMT, surface and underwater swimming times improved markedly after RRMT-5 and RRMT-3. The improvements in endurance performance were not due to changes in the aerobic fitness of the subjects as this was constant over the entire period (RRMT and RRMT-M). The improved swimming endurance was also, in all likelihood, not due to psychological factors as previous studies in divers have shown that placebo RMT did not have an impact on respiratory or fin swimming performance and RMT studies on land have shown that control and placebo groups did not improve performance.

The  $\text{PI}_{\text{max}}$  and  $\text{PE}_{\text{max}}$  increased significantly after both RRMT-3 and RRMT-5, and appear to have led to increased tidal volumes during exercise (11 to 14% in conjunction with a 20-10% decrease in  $f_b$  for RRMT-5 and RRMT-3 respectively). It has been found that training that incorporated 30 inspiratory efforts against a resistance  $\sim 50\%$  of maximal inspiratory mouth pressure, similar to that used in the present study, resulted in a  $V_T$  during land exercise that was maintained during the latter stages of incremental exercise, versus a more tachypneic pattern in the placebo group, as was also observed in the present study during the pre-RRMT testing. It is possible that the increased inspiratory strength and/or endurance can contribute to a shortened inspiration time, however this was not measured in the present study. Such a change, in conjunction with the increased strength and endurance of the respiratory muscles, might allow a more optimized (increased) post-expiratory muscle length and lung volume making the muscles better capable of performing respiratory work as well as reducing flow resistance (due to the increased lung volume) thereby minimizing the work of breathing.

The adaptations described above may reduce the oxygen cost of ventilation. In the present study, total ventilation swimming underwater decreased more than 10% and the steady-state  $\text{VO}_2$

decreased by 8%, as well. Given the energy cost of ventilation previously reported (5) it is attractive to propose that the post-RMT reduction in  $\dot{V}O_2$  as due to the reduction in  $\dot{V}_E$ . This conclusion is consistent with reported increases in oxidative enzyme potentials (SDH, citrate synthase) in the diaphragm and intercostals muscles and increased proportion of Type I fibers in external intercostals following respiratory loading.

The work of breathing during maximal and submaximal exercise is the primary determinant of respiratory muscle blood flow. Respiratory muscle  $\dot{V}O_2$  increases linearly with ventilation, up to the ventilatory threshold, after which a marked increase in ventilation occurs. When exercise is performed (during terrestrial conditions) at 70%  $\dot{V}O_{2\max}$ , the respiratory muscles require ~ 5%, and at maximal exercise ~10%, of the total  $\dot{V}O_2$ . Due to the high ventilation during exercise above  $\dot{V}O_{2\max}$  the  $\dot{V}O_2$  the higher resistive and elastic respiratory work may require nearly 15% of total  $\dot{V}O_2$ . A high  $O_2$  cost of ventilation may especially be the case in diving where trans-thoracic hydrostatic pressure differences (static lung loading), breathing gear resistance and gas density increase the work of breathing. During such increased work of breathing and resultant high  $O_2$  demands by the respiratory musculature its need for increased blood flow is likely to compromise the blood flow, and thus  $O_2$  delivery, to the skeletal locomotor muscles. Increased diaphragmatic blood flow along with decreased limb locomotor blood flow during submaximal exercise have been reported after increasing the work of breathing in rats via experimental congestive heart failure.

Training three days per week was as effective as five days per week suggesting that the more intense schedule may have caused overtraining. Studies of other skeletal muscles have shown that full strength gain could only be demonstrated after 4 days, as compared 2-4 days of rest from training. Thus, daily training may not allow sufficient recovery. Therefore, post training fatigue can give misleading information about the ability of RMT to enhance exercise endurance. This is illustrated by the outcome of a study which failed to show an ergogenic effect of RMT on terrestrial locomotion when tested one day after intense RMT. Additionally, it has been shown that testing about five days after RMT reveals greater improvement than testing one day after RMT. Other studies of high intensity aerobic training and resistance training have shown that significant improvements can be made in four to five weeks, as has previously been demonstrated for surface swimming.

Dr. Linholm also participated in another study to examine the prevalence of low  $CO_2$  sensitivity in divers, and the potential role if respiratory muscle training in "normalizing"  $CO_2$  sensitivity. Alveolar ventilation is typically tightly regulated to hold  $P_{aCO_2}$  at 40 mmHg in normal healthy subjects. However, in certain situations a person may hyper- or hypo-ventilate relative to a given  $CO_2$  production, resulting in a decrease or increase in  $P_{aCO_2}$ , respectively. One situation conducive to  $CO_2$  retention is diving with Self Contained Underwater Breathing Apparatus (SCUBA) which is a common professional, military and sport activity. SCUBA diving involves immersion, exercise, and increased work of breathing due to hydrostatic pressure and airway resistance, all of which expose the respiratory system to increased work of breathing. Voluntary hypoventilation (so called "skipped breathing"), routinely practiced by some divers, may cause hypercapnia. Hypoventilation may also occur due to inability to perform the increased respiratory work required in the physically unique diving environment. Diving-induced  $CO_2$  retention merits investigation because of the potential for  $CO_2$  narcosis and its tendency to enhance central nervous system oxygen toxicity

(CNS-OT) as well as nitrogen narcosis. Carbon dioxide retention alone may incapacitate a diver due to the narcotic effects of CO<sub>2</sub>, a phenomenon originally termed shallow water blackout as observed in oxygen breathing divers when the CO<sub>2</sub> scrubbers in their breathing gear functioned poorly. It has also recently been shown in animals that even mild hypercapnia can increase the risk of CNS-OT when hyperoxic gas mixtures are breathed. It has furthermore been reported that CO<sub>2</sub> retaining divers may convulse while still within what is generally considered to be the safe limits for hyperoxic exposure.

The mechanisms behind CO<sub>2</sub>-retention are still unclear, but one factor involved in hypoventilation in divers may be low respiratory CO<sub>2</sub> sensitivity. Immersion *per se* does not alter CO<sub>2</sub> sensitivity in either divers or non-divers. Carbon dioxide sensitivity has been reported to be the same at rest and during exercise. It has been shown in a few cases that high external breathing resistance may cause CO<sub>2</sub> retention even without prodromal symptoms (no dyspnea). It has also been suggested that the ability to tolerate high CO<sub>2</sub> enables working divers to function when breathing becomes cumbersome, an "adaptation" with risks since nitrogen narcosis at depth is additive to carbon dioxide narcosis, and the combined effects may quickly incapacitate a diver.

It is also possible that part of the hypoventilation in divers is a conditioned behavior learned by divers; it has been shown that divers and ex-divers, who had not been diving for years, hypoventilated compared to a normal population when subjected to an exercise test. However, it was not determined whether this was an adaptation or was due to selection/genetics in the divers. If hypoventilation in divers is due to an acquired behavior it could possibly be normalized by training with a paced breathing pattern. Another potential cause of hypoventilation in divers is inadequate respiratory muscle performance due to poor function or fatigue which may attenuate the CO<sub>2</sub> response and lead to CO<sub>2</sub> retention. Studies have shown that RMT can improve respiratory muscle function and reduce swimming fatigue.

We hypothesized that respiratory sensitivity to CO<sub>2</sub> quantified with a standard rebreathing test would be normalized by RMT in non-immersed subjects with low CO<sub>2</sub> sensitivity. This was a retrospective study that analyzed CO<sub>2</sub> sensitivity data collected, and not yet reported, during three previous studies that were specifically designed to determine if RMT improved respiratory muscle and swimming endurance in 35 male certified SCUBA divers. Data were pooled from these three studies even though the exact protocol of the RMT varied slightly.

The present study indicates that low respiratory CO<sub>2</sub> sensitivity may be normalized by training of the respiratory muscles using paced breathing patterns that specifically enhance respiratory muscle performance as tested by respiratory muscle endurance during isocapnic hyperventilation or by maximal inspiratory and expiratory pressures. Despite years of research, no single explanation has been offered for divers' CO<sub>2</sub> retention and no test has been shown to reliably explain or predict exertional hypoventilatory hypercapnia (CO<sub>2</sub>-retention) in divers, which suggests that this phenomenon could be of multifactorial etiology. Divers' CO<sub>2</sub> retention is probably influenced by increased hydrostatic pressure across the chest wall (static lung loading), gas density and composition, and by exercise hyperpnea, all of which may cause increased work of breathing. Additionally, lacking capacity of the respiratory muscles to perform work as well as conditioned behavior may contribute to CO<sub>2</sub> retention.

During rest and submaximal exercise in air,  $V_E$  is typically proportional to  $V_{O_2}$  and  $V_{CO_2}$  while the  $P_{aCO_2}$  is held relatively constant. In SCUBA divers however, it has been shown that ventilation may not match  $CO_2$  production resulting in an increase in  $P_{aCO_2}$  (hypoventilation) and consequent  $CO_2$  retention. Remarkably, Young (20) found that excessive respiratory  $CO_2$  sensitivity before navy diving training might be a predictor of failing the diving course. Moreover, it was found that trainees who passed the course exhibited a reduced  $CO_2$  sensitivity at the end of it. That diving-related changes in respiratory pattern may be long-lasting is suggested by the findings that showed that divers and even ex-divers who had not been diving for years hypoventilated compared to a normal population when subjected to an exercise test. Divers who use open circuit systems are constantly encouraged by experience to reduce breathing since every breath changes the divers buoyancy, thus a relatively slow breathing is more comfortable. Also, a novice diver often uses a lot of air, probably due to inexperience with buoyancy technique and a higher stress level and less efficient movements in the water. Although discouraged by instructors, scuba divers often compare their air consumption as a means of evaluating diving skill with the resulting motivation to reduce breathing (a.k.a. "skipped breathing") while at depth.

Reduced  $CO_2$  response, hypoventilation, increased end-tidal  $CO_2$  and ensuing hypercapnia have been observed in escape tank instructors, trained underwater swimmers, and non-immersed divers in response to exercise while breathing air as well as  $O_2$ . These blunted responses are not universally seen since some studies report that  $P_{CO_2}$  is defended during diving. The apparent disagreements between studies may be due to individual diver differences or diver training and experience with some divers defending a relatively physiological  $P_{CO_2}$  (at the expense of suffering dyspnea) during actual diving and others allowing the  $P_{CO_2}$  to rise without much discomfort. Similar tendencies were observed in the present study of respiratory  $CO_2$  sensitivity with one-half of the subjects defending  $P_{CO_2}$  while the other allowed it to rise prior to RMT. This suggests that the individual differences in respiratory responses to diving may be due to differences in  $CO_2$  sensitivity.

As for an explanation of the effect of RMT to modify the  $CO_2$  sensitivity of the subjects who were either high or low responders it is highly likely that the mechanism was one of changing the effector side of the reflexive respiratory control loop, i.e. the motor response of the respiratory muscles to a given chemoreceptor stimulation. The fact that the  $CO_2$  sensitivity was unchanged by RMT in subjects whose response was normal before RMT is consistent with the notion of a feedback from the effector system to the respiratory center's response to rising  $P_{CO_2}$ .

It is important to compare RMTE and RMTS in sufficiently large groups of subjects so as to be able to determine if one mode of training is more effective than the other. A related question, also to be studied, is whether, after a certain training effect has been obtained, it can be maintained with a lower intensity "maintenance training". Preliminary results in runners, in our laboratory, suggest that this may be a fruitful line of study. Moreover, while current RMT schedules were based on what was, from early exploratory studies, known to work well, no systemic study has been performed to define the "dose/response" relationship between RMT effort and improvements in physical endurance. This relationship has important practical implications for the end user. Earlier preliminary studies, performed in house, suggest that RMT may change a subject's ventilatory  $CO_2$ -sensitivity. Because of the important safety aspects of divers'  $CO_2$  elimination, the  $CO_2$ -sensitivity

will be studied in this new, larger subject population. Moreover, because the exercising diver runs the greatest risk of excessive CO<sub>2</sub> accumulation (hypercapnia), the CO<sub>2</sub> sensitivity tests will be performed in subjects while they are swimming under water as well as during rest.

Early studies have indicated that maximal exercise is limited by the build up of lactic acid (La) and leads to muscle fatigue. The time it takes for fatigue to occur is dependent on the exercise intensity and the maximal aerobic power of the individual subject (VO<sub>2max</sub>) as these factors determine the rate of La buildup. In trained subjects, La increases above resting levels with time (La) when exercise intensity reaches 70-80% of VO<sub>2max</sub>, with higher La at higher intensities. At higher exercise levels (80-90% VO<sub>2max</sub>) maximal cardiac output and delivery of blood to locomotor muscle is achieved and there is a heightened respiratory stimulation (increased V<sub>E</sub>/VO<sub>2</sub>) due to the build up of lactic acid. It has been shown that the work of breathing at the higher exercise intensities affects exercise performance. The mechanism behind the reduced performance is that, at high exercise intensities, there is an increased need for oxygen delivery to respiratory muscles that cannot be accomplished by an increase in cardiac output as it is at or near maximal levels. The increased oxygen delivery to respiratory muscles is achieved by a reduction in blood flow to exercising locomotor muscles (LMBF) while the blood flow to respiratory muscles increases (RMBF), leading to reduced oxygen delivery and increase La and La. The stress on the respiratory muscles and reduced oxygen delivery to locomotor muscles cause fatigue in both types of muscles.

Fatigue at exercise levels below 70-80% of VO<sub>2</sub>, where La does not increase as a function of time, is thought to be limited by the availability of fuel for oxidation (carbohydrates or fats) (Pendergast 2000). As the cardiac output and MBF are submaximal, it is assumed that there is no limitation of oxygen delivery to locomotor or respiratory muscles. It has been shown in short-term submaximal experiments that the work of breathing is low and cardiac output, and LMBF are not affected or limiting. However, RMTE can significantly improve endurance cycling or running time, without changes in cardiovascular function. This suggests that other factors, modifiable by RMT, are playing a role in limiting endurance performance. In another study, strength RMT and endurance RMT were studied over a five-week period, with a 26% improvement in cycling endurance time in trained cyclists. The HR, V<sub>E</sub> and venous blood lactate were unaffected by either RMTS or RMTE, however their measurements were taken in the steady-state period prior to respiratory muscle fatigue. It is clear that during sustained submaximal exercise there is a decrease in plasma volume and increased perfusion of the skin, which could alter the distribution, if not the level, of cardiac output. It is our hypothesis that during sustained exercise, as the run progresses, there is an increase in the anaerobic component of energy production, and that La will increase as a function of time. The increase in the anaerobic component, as reflected in blood lactate levels, may be caused by a reduced muscle blood flow and oxygen delivery (St Croix 2000) and/or an increased reliance on fast twitch muscle fibers that are dependent on glycogen and produce La. The changes in muscle and blood pH lead to hyperventilation and eventually respiratory and locomotor muscle fatigue. We propose that RMT can delay the onset of anaerobic metabolism in both locomotor and respiratory muscles and the reduced muscle blood flow and hyperventilation. Taken together, the delay in onset of these physiological derangements would lead to a prolonged exercise time. In fact, RMT has been shown to increase exercise endurance time at submaximal VO<sub>2</sub> levels.

The effect of environmental stress, including cold or hot water and breathing resistance of UBA would exacerbate the locomotor and respiratory muscle fatigue as these stresses have been shown to

reduce blood flow and oxygen delivery, increase anaerobic metabolism and lead to hyperventilation and fatigue. Studies of exercise training of locomotor muscles have shown that the fatigue can be delayed by training as the oxygen delivery is increased by both increased blood flow and extraction of oxygen, which leads to a delay in the anaerobic component and hyperventilation. The purpose of this aspect of this proposal will be to test the hypothesis that during endurance exercise at about 70%  $\text{VO}_{2\text{max}}$  (at or above the anaerobic threshold) there is a buildup of  $\text{La}$  that after a time (about 40-60 min) leads to a hyperventilation that increases respiratory work. It is further hypothesized that RMTE and RMTS would reduce the rate of  $\text{La}$  buildup during the early part of endurance exercise, delaying the increased respiratory work and prolonging the exercise time prior to hyperventilation and locomotor and respiratory muscle fatigue.

The data collected to date comparing strength and endurance RMT to a placebo RMT training has shown that endurance time increased significantly in both strength RMT and endurance RMT (91 and 59%, respectively), but not in the placebo group. Heart rate and ventilation significantly decreased by 12% and 13%, respectively, only after endurance RMT. MVV, SVC, FVC and FEV1 did not change significantly in any group. However respiratory endurance increased 27% and 212% after strength RMT and endurance RMT, respectively. Maximal pressures and  $\text{CO}_2$  sensitivity were not affected by RMT. In summary, endurance RMT improved respiratory endurance and both endurance RMT and strength RMT improved fin swimming endurance, but only endurance RMT decreased heart rate and ventilation. It is concluded that RMT is effective in improving swimming endurance, and this was not a placebo effect. It is speculated that RMTE will have greater positive effects than RMTS on divers' swimming endurance when using UBA. However, strength RMT has greater improvement at this stage of the study. Finally, extrapolating from the well known fact that training of locomotor muscle is effective, if employed three times per week, and can be maintained by two training sessions per week, we propose to investigate whether RMT performed three times per week will offer significant benefits to the diver, and if twice per week could maintain the improvements. If this were true, divers could train more efficiently and RMT would prove more practical to Navy divers.

As a follow up to previous studies in CRESE showing the benefits of respiratory muscle training on pulmonary and exercise performance at the surface and 4 fsw, studies were designed and carried out by Dr. Ray to investigate if the improvement were greater at depth where the work of breathing is greater. In the past, the pulmonary system was not considered to be a limiting factor to exercise performance in healthy individuals. However, it is well known that a reduction in gas transport and respiratory muscle weakness limits exercise performance in patients with chronic obstructive pulmonary disease (COPD) and more recently, respiratory muscle weakness has also been shown to reduce sub-maximal as well as maximal exercise performance in healthy individuals. Because of the high ventilatory demands associated with maximal exercise, respiratory muscles compete with locomotor muscles for blood flow. In general the respiratory muscles account for 4% of the total oxygen consumption but that number can increase to 10-15% during maximal exercise and when there is airflow limitation. As exercise intensity increases, type III-IV afferent nerves in the respiratory muscles are stimulated, resulting in respiratory muscle vasodilatation and lower extremity vasoconstriction.

In comparison to exercise on land, pulmonary mechanics underwater are severely challenged due to the hydrostatic pressure differences across the chest wall and the added resistance associated with

breathing from a self contained underwater breathing apparatus (scuba). Because of the progressive increase in gas density at greater depths as well as the effects of immersion on the respiratory system, diver's experience increases in airflow resistance, both at rest and during exercise. The similarities between divers, athletes, and patients with lung disease are that their ventilation may become flow limited during high-intensity exercise which makes them vulnerable to an elevated work of breathing and premature fatigue of the respiratory muscles.

Leith and Bradley (1976) were the first to demonstrate improvements in respiratory muscle strength and endurance following specific respiratory muscle training protocols. Since then, multiple studies have shown improvements in running, cycling and rowing performance following respiratory muscle training and more recently, it has been shown to improve fin-swimming performance at the surface and at 4 feet of water (fw). That study also demonstrated that a resistance training protocol was more effective at improving respiratory muscle strength and swimming performance than a voluntary isocapnic hyperventilatory (endurance) protocol (66% vs. 26% improvement of exercise endurance, respectively).

The purpose of the current study was to evaluate whether resistance respiratory muscle training (RRMT) would improve respiratory muscle strength and fin-swimming performance at greater depth (55 fw, 2.67 ATA, 270.5 kPa), where gas density and hence the work of breathing are significantly increased. Based on previous finding from our laboratory at 4 fsw it was hypothesized that RRMT will: 1) reduce respiratory muscle limitations, 2) increase respiratory muscle strength immediately following an endurance swim trial and 3) improve fin-swimming endurance at 55 fsw.

The primary findings from this study are that RRMT: 1) demonstrated that improvements in respiratory muscle performance lead to increased fin-swimming performance at depth (55 fw) where the work of breathing is significantly elevated, 2) resulted in a decrease in  $V_E$ ,  $f_b$  and  $\dot{V}CO_2$  and presumably  $\dot{V}O_2$ , 3) attenuated the decline in  $V_t$  observed during the pre-RRMT test, 4) was not connected with the tachypnea seen towards the end of exercise during the pre-RRMT test. Fin-swimming endurance at depth was improved by ~ 60% following respiratory muscle training and we suggest that the improvements are primarily related to respiratory muscular adaptations following training. The potential independent effects of fin-kicking technique, aerobic capacity or leg strength were most likely eliminated in the present study by the four wk. fin-training protocol performed prior to and during the respiratory muscle training protocol. Therefore, based on the information from the current study and from our previous work, we suggest that fin-swimming ability was not a co-variate explaining the improvements in underwater exercise performance. In addition, because the use of placebo control groups in previous studies has shown that sham-RMT has no effect on performance we feel confident that the increase in performance we observed was not related to psychological factors, a notion strongly supported by the significantly greater respiratory muscle strength in the "stop" trials post-RRMT than in the equally long-lasting open ended swims pre-RRMT.

Prior to RRMT, the increase in  $V_E$  and  $f_b$  combined with a decrease in  $V_t$  at the end of exercise as well as the reduced ability to generate  $P_E$  max and  $P_I$  max post-exercise suggests respiratory muscle fatigue may have been a limiting factor during the fin-swimming endurance test. Ventilation is known to be tightly correlated to  $\dot{V}O_2$ , however, in the presence of metabolic acidosis caused by

anaerobic glycolysis and lactic acid accumulation,  $\dot{V}_E$  is driven up out of proportion to  $\dot{V}CO_2$  (respiratory compensation for a metabolic acidosis). Although lactic acid was not measured, we suggest that the tachypneic ventilatory pattern at the end of exercise pre-RRMT is consistent with an increase in lactic acid and may have induced premature respiratory muscle fatigue. Based on the discussion above and the data from the present study we suggest that during the last min of exercise the energy cost of respiration was elevated pre-RRMT as the subjects hyperventilated, which by contrast they did not do post-RRMT.

At workloads below 70% of  $VO_{2max}$  the respiratory muscles require around 4.5% of the total  $VO_2$ , while, at maximal exercise, that value may be 10% of the total oxygen consumed and may even approach 15% in the presence of expiratory airflow limitation at 1 ATA. The elevated energy costs associated with breathing during sub-maximal and maximal exercise influences respiratory and locomotor muscle blood flow. To accommodate the increased metabolic demands during maximal exercise, type III-IV afferent nerves in the diaphragm and other respiratory muscles are reflexively stimulated resulting in vasodilatation and increased blood flow to the respiratory muscles and vasoconstriction and decreased blood flow to the leg muscles. This may have occurred pre-RRMT, but not post-RRMT in the present experiments.

When exercising under water, divers are often exposed to a negative static lung load (the pressure difference between the outside of the chest compared to the alveolar air pressure) and increases in gas density and airflow resistance associated with the divers' under water breathing apparatus place additional demands on the respiratory muscles at rest and during exercise. Collectively, these changes are, in all likelihood, conducive to respiratory muscle fatigue and limiting to underwater exercise performance. Similar to what was the case during the fin-swimming endurance test pre-RRMT,  $\dot{V}_E$  was elevated from rest and remained so throughout the entire exercise test following training. The post-RRMT ventilatory response was less pronounced and the hyperventilatory response was eliminated during the last minutes of exercise, although the divers exercised significantly longer. Even with the decreased ventilatory response and longer exercise time,  $V_t$  was similar to the pre-RRMT volumes during the first 20 min of exercise and tended to decline more gradually and less pronounced following RRMT. Previously we have demonstrated that RRMT increases  $V_t$  at the surface and at 4 fw. The primary difference between the current study and the previous one is that the current study was performed at a depth that significantly impacts pulmonary mechanics and the work of breathing because of the increase in gas density. Although  $V_t$  did not increase following training, RRMT prolonged the time and blunted the decline in  $V_t$  during the endurance swim.

The current study utilizes the subjects' ability to produce maximal inspiratory and expiratory pressures following the swimming trials as an indirect measure of respiratory muscle fatigue. Whereas measuring the ability to generate maximal pressures immediately after, rather than before, the endurance test is a more functional approach towards establishing respiratory muscle fatigue as a limiting factor during underwater fin-swimming. Following RRMT and before the swimming endurance tests,  $P_E$  and  $P_I$  max were non-significantly increased 14% and 18%, respectively. When measured during the endurance swims for the same time post-RRMT (post-stop), the ability to generate maximal pressures were significantly increased ( $P_E = 29\%$  and  $P_I = 88\%$  respectively,  $p < 0.05$ ). These results demonstrate that RRMT blunted the decrease in strength observed Pre-RRMT, and the development of respiratory muscle fatigue. Following the open-ended post-RRMT

endurance swim where the times were longer,  $P_E$  max and  $P_I$  max were not significantly different post-RRMT compared to pre-RRMT, indicating a similar loss of strength and fatigue.

The reductions in total and alveolar ventilation in the present study were consistent with a reduced  $\dot{V}_{CO_2}$  following training and we assume there was a similar drop in  $\dot{V}O_2$ , although it was not measured; such a change was observed in a previous study using an identical protocol. Because RRMT is not expected to affect substrate utilization in fin-kicking muscles and did not change exercising heart rate, we suggest that the reductions in  $\dot{V}CO_2$  and  $\dot{V}O_2$  may be the result of respiratory muscle adaptations following RRMT. It is possible that, despite performing a resistive strength training protocol, the respiratory muscles became more efficient and adapted to the increase in airflow resistance as a result of training. In a study by Gea et al. it was demonstrated that 4 days of intermittent inspiratory loading ( $\sim 80$  cmH<sub>2</sub>O) in dogs increased the expression of the slow myosin heavy chain isoform in the costal and crural diaphragm as well as the intercostal muscles (35). An increase in the slower myosin heavy chain isoform may be an adaptation to the increase in airflow resistance and thus, improve respiratory muscle and exercise endurance, especially under conditions of airflow limitation. A similar change in myosin has also been reported in patients with COPD, a condition with continuous airflow limitation similar to swimming at depth.

The current study extends our previous findings from experiments at 4 fw and at the surface to include improved fin-swimming performance at 55 fw. The improvements in respiratory muscle strength were sufficient to compensate for the added resistance from the increase in gas density as the improvements in endurance time were similar to those at 4 fw. These findings of respiratory muscle training improving exercise performance in divers are supported by previous studies in runners, cyclists and rowers. The current study demonstrates that respiratory muscle fatigue may be the performance limiting factor during underwater fin-swimming and we conclude that RRMT prolongs fin-swimming endurance possibly through respiratory muscular adaptations following training that may reduce the work of breathing and/or improve respiratory muscle efficiency, particularly towards the end of exercise, thus reducing the respiratory muscle blood flow requirements and the "stealing effect" from the fin-kicking muscles. Although the current study cannot distinguish one from the other and therefore, further studies are required to address each mechanism individually.

Fatigue of the respiratory muscles was not historically believed to limit exercise performance, however, studies have shown reductions in sub-maximal and maximal exercise secondary to respiratory muscle fatigue while multiple studies have shown improvements in running, cycling and rowing performance following a specifically designed respiratory muscle training protocol. Previously we have used resistance respiratory muscle training (RRMT) to increase respiratory muscle and swimming performance at increased depths and pressures where the work of breathing is significantly elevated.

Leith and Bradley (1976) were the first to show improvements in respiratory muscle strength and endurance following specifically designed respiratory muscle training protocols. Their original study improved respiratory muscle strength ( $\sim 55\%$ ) but not endurance using a protocol requiring 100% of static inspiratory and expiratory muscle strength; while a specific respiratory muscle endurance protocol increased respiratory muscle endurance but not strength. More recently, we

used a strengthening protocol that required the subject's to generate a much smaller percentage of maximal pressure (~40% max) to increase respiratory muscle strength. Despite training at a lower percentage of maximum our subjects were able to significantly increase inspiratory and expiratory muscle strength 12 and 14% respectively. Clearly, the improvements in respiratory muscle strength were not of the same magnitude as Leith and Bradley's, however, the subjects from our previous studies also demonstrated an increase in respiratory muscle endurance (~8-10%). As a result, training at such a high intensity (100% of max) increases respiratory muscle strength while sacrificing any potential gain in respiratory muscle endurance; whereas training at intensities less than maximum leads to improvements in respiratory muscle strength and endurance, a feature that may be essential to improvements in exercise performance at depth.

In comparison to exercise on land, the respiratory system underwater is severely challenged due to the hydrostatic pressure differences across the chest wall in addition to breathing from a self contained underwater breathing apparatus (SCUBA). Because of the progressive increase in gas density at greater depths, diver's experience increases in airflow resistance and the work of breathing at rest and during exercise. In addition, pulmonary mechanics are altered because of the effects of immersion on the respiratory system or to changes in the static lung load (SLL); the differences in the hydrostatic pressure surrounding the chest and the pressure at the mouth. The underwater challenges to the respiratory system increase the likelihood of developing respiratory muscle fatigue even at rest.

The physiological adaptations associated with underwater swimming and SLL impose additional demands on the divers' respiratory system, demonstrated by changes in end expiratory lung volume (EELV) at rest and during exercise. Lundgren et. al. (1984) studied EELV while exposed to different static loads ranging from -30 to +30 cm H<sub>2</sub>O. From that study, a negative SLL was associated with a reduced EELV at rest, with a slight but non-significant increase in EELV during exercise. The implications associated with altering EELV is that breathing at a lung volume that represents either a lower or higher percentage of total lung capacity, i.e. at a decreased or increased EELV, places the respiratory muscles at a less than optimal position on their length-tension curve and reduces their ability to generate and maintain adequate force. Therefore, it remains to be determined if stronger respiratory muscles can attenuate the immersion and negative SLL effects on the respiratory system and negate the decrease in EELV, reducing the development of respiratory muscle fatigue at depth?

The mechanisms of why and how RRMT increases sub-maximal swimming performance are unknown and probably multifactorial. As a result, the purpose of the current study was to evaluate whether a progressive resistance respiratory muscle training (RRMT) protocol would improve respiratory muscle strength and fin-swimming performance at greater depths (120 feet of sea water, 4.64 ATA, 469.78 kPa) and to test whether the improvements in swimming performance are correlated to improvement in pulmonary mechanics (EELV) or to a reduced work of breathing, or both following training. It is hypothesized that RRMT will: 1) increase respiratory muscle strength, 2) increase sub-maximal swimming time, 3) reduce the work of breathing, and 4) improve pulmonary mechanics (EELV) by altering lung volumes.

The present study demonstrates that RRMT improves sub-maximal underwater swimming performance at increased depths (120 fsw) where the work of breathing is significantly increased.

The improvements in exercise performance are associated with 1) increases in respiratory muscle strength and endurance, 2) reduced total ventilation and breathing frequency while maintaining tidal volume throughout the duration of the endurance swim, 3) a tendency to decrease the work of breathing and 4) changes in pulmonary mechanics cannot account for the improvements in sub-maximal swimming performance because exercising lung volumes did not change following RRMT.

The current study adds potential mechanisms explaining why respiratory muscle training improves sub-maximal underwater swimming performance. In previous studies we have shown RRMT to increase swimming performance at the surface, at 4 fsw and at 55 fsw (33%, 66%, and 60%, respectively). From the current study, the improvements in swimming performance (87%) as well as the gains in respiratory muscle strength far out seed those obtained in our previous work. In two previous studies from our laboratory using the identical training protocol to each other, demonstrated a 10-15% increase in respiratory muscle strength following training. Both of these studies required the subjects to inspire and expire against a set pressure (50 cmH<sub>2</sub>O) that did not change throughout the duration of the study and equal to 40-43% of the subject's baseline maximal pressures. In contrast the current study used a progressive resistance training protocol, such that the mouth piece resistance (inspiratory and expiratory) was increased ~10 cmH<sub>2</sub>O each week over the four week training period, representing a training intensity that was equal to 31-65% (range 42-74 cmH<sub>2</sub>O) of their initial baseline maximal pressures. As a result, the progressive training protocol was associated with a much greater increase in the subject's ability to generate maximal pressure (inspiratory and expiratory). The additional gains in strength may account for the superior swimming performance compared to our previous studies and may explain why the constant training stimulus did not have the same impact on swimming performance at 55 fsw compared to swimming performance at 4 fsw? As a result, it is suggested that stronger respiratory muscles are more important at increased depth where gas density and the work of breathing are increased; such that the stronger the muscle the greater the improvement in swimming performance.

Respiratory muscle strength improved quickly and continued to increase throughout the four week training period. A similar paper showed a continual increase in respiratory muscle strength for 2-3 months following four weeks of a constant resistance protocol, despite the fact that the frequency of training was reduced from three to two days per week. Because the current improvements in strength were so much greater than our previous studies it is unknown if, where or when a plateau in strength would have occurred and justifies the need for additional studies investigating the relationship between resistance training and the respiratory muscles. Moreover, the large increase in strength may explain the improvements in slow vital capacity.

In contrast to our previous studies, the current study also demonstrated extraordinary gains in respiratory muscle endurance. Using a strength protocol to increase respiratory endurance is in contrast to original RMT studies. Leith and Bradley (1976) demonstrated that using a maximal resistance protocol (isometric) increases respiratory muscle strength but not endurance, while, a protocol designed to increase respiratory muscle endurance improves respiratory muscle endurance but not strength (Leith and Bradley 1976). Even with the progressive training protocol the training intensity never surpassed 50% of the previous week's maximum pressures; however, it was sufficient to increase respiratory muscle strength, endurance and exercise performance. As a result,

improvements in respiratory muscle endurance may be just as important as increases in strength to enhance underwater swimming performance.

Ventilation was stable for the majority of the endurance test, but tended to decrease during the last minute of exercise. The drop in ventilation was associated with a non-significant reduction in tidal volume, a ventilatory response that is in contrast to our previous work but possibly indicative of premature respiratory muscle fatigue. Typically, the last minute of exercise is associated with an increase in ventilation or a tachypneic response above what the oxygen demands are for the body. The tachypneic response is primarily driven by an increase in breathing frequency while tidal volume is maintained or reduced. This response known as a respiratory compensation for a metabolic acidosis is generally associated with elevated respiratory and locomotor muscle lactate, respiratory muscle fatigue and is normally associated with the termination of exercise. Because the subjects were unable to increase breathing frequency during the last minute of exercise, it is possible that the challenges associated with the increased depth and pressure severely challenged the respiratory muscles, enhancing the development of respiratory muscle fatigue and limiting exercise performance.

The underwater environment severely challenges the respiratory muscles by imposing heavy respiratory loads. In comparison to exercise on land, pulmonary mechanics underwater are severely challenged due to the hydrostatic pressure differences across the chest wall in addition to the added resistance associated with breathing from a self contained underwater breathing apparatus (scuba) (Lundgren 1984). Because of the progressive increase in gas density at greater depths as well as the effects of immersion on the respiratory system, diver's experience increases in airflow resistance, both at rest and during exercise. Moreover, exercising at 120 fsw is associated with breathing gas that is 4-5 times denser than the air we breathe at sea level, thus the increased gas density requires additional energy requirements during exercise especially with increased ventilation, potentially explaining why the respiratory system was unable to increase breathing frequency towards the end of exercise.

The increase in respiratory muscle strength following RRMT helped improve swimming endurance. Despite swimming for a longer period of time, the post-RRMT ventilatory response was reduced for the duration of the swimming test when compared to the pre-RRMT endurance test. This response, consistent with our earlier work, is associated with reduced minute and alveolar ventilation, carbon dioxide production and a slightly reduced breathing frequency, suggesting a reduced energy cost of breathing. Although not measured in the current study we have previously shown a decrease in oxygen consumption following training, which may have been correlated to the reduced ventilation. Given that the energy cost of respiration is 4-5% of the total body oxygen consumption and can reach as high as 10-15% in the presence of airflow limitation, a reduction in the respiratory muscle energy requirements will decrease the "stealing phenomenon" of blood from the locomotor muscle to the respiratory muscles and prolong exercise. Consequently, the reduced ventilation may be associated with a more efficient respiratory system and/or a reduced work of breathing.

The increased work of breathing underwater can be explained by the transient increase in pressure and gas density associated with increasing depth. Combined these changes alter pulmonary mechanics (lung volume) and increase airflow resistance (ref). We hypothesized that stronger respiratory muscles would offset the environmental challenges and decrease the work of breathing

while prolonging exercise time. As a result of training, there was a tendency for the total work of breathing to be reduced. More specifically, the slight reduction was associated with a decrease on the inspiratory side while expiratory work did not change. However, when expressed in joules per liter the differences were negated. Even though the respiratory muscles were stronger and potentially more efficient, the reduced work of breathing was more likely associated with the reduced respiratory rate and it is therefore possible that the reduced respiratory rate is the reason why oxygen consumption is reduced following RMT demonstrated that four days of intermittent inspiratory loading ( $\sim 80$  cmH<sub>2</sub>O) in dogs increased the expression of the slow myosin heavy chain isoform in the costal and crural diaphragm as well as the intercostal muscles. An increase in the slower myosin heavy chain isoform may be an adaptation to the increase in airflow resistance and thus, improve respiratory muscle and exercise endurance, especially under conditions of airflow limitation. A similar change in myosin has also been reported in patients with COPD, a condition with continuous airflow limitation similar to swimming at depth. Thus, it is possible that the stronger respiratory muscles become more efficient secondary to the reduced respiratory rate and therefore, blood flow to the locomotor muscles may be maintained, reducing the "stealing effect" during exercise.

Stronger respiratory muscles are important at increased pressure but may not directly decrease the work of breathing. It is possible that the stronger respiratory muscles indirectly improve performance by altering pulmonary mechanics, i.e. changing lung volume or expiratory reserve volume (ERV) during submersed exercise. Lung volumes underwater are determined by the interaction between internal and external forces, including respiratory muscle tone and thoracic blood volume. While submersed and exposed to a progressively larger negative static lung load (pressure around the chest is greater than the pressure at the mouth), ERV is transiently reduced, potentially exposing the divers to an increase in airflow resistance. However, Thalmann et. al. (1979) showed a gradual increase (non-significant) in ERV from rest to sub-maximal exercise while exposed to increased pressure and a negative SLL, suggesting the potential to overcome the environmental challenges with a stronger respiratory muscle. Despite stronger respiratory muscles, it seems they are still not capable of changing lung volumes during sub-maximal exercise; however this does not counteract the benefits of RRMT. During negative pressure breathing the respiratory muscles may develop greater force for the same level of neural activation and once strengthened this response may be improved. As a result, an increase in force for a given level of neural activation may explain the increase in tidal volume following training. In addition, the training requires the subjects to perform vital capacity maneuvers against a known resistance, therefore strengthening the respiratory muscles at all lung volumes and muscle lengths that may be compatible to the changes in lung volumes with immersion.

It is concluded that RRMT improves respiratory muscle strength, endurance and swimming performance underwater, a response that is consistent with our previous work. Based on the above information, the work of breathing was slightly but non-significantly reduced and the improved strength did not alter pulmonary mechanics and therefore, cannot explain the improvements in performance. It seems reasonable to suggest that the adaptations to the muscles are associated with improvements in muscle efficiency and that is what may be responsible for reducing oxygen consumption and the mechanical efficiency of the respiratory system following RRMT.

## CONCLUSIONS:

**Professional Development:** The conclusion for this ONR Postdoctoral Fellowship can be summarized in three results. First both Drs. Lindholm and Ray were trained to the point of obtaining independent university positions, published papers, participated in grant writing and succeeded in teaching in the Physiology Department. Based on their development we can conclude that the main objective of this Fellowship was met.

**Breath-holding:** In addition scientific contributions were made in two areas of undersea medical research, also an objective of this ONR Postdoctoral Fellowship. In the area of breath-hold diving three major conclusions could be drawn. First, fasting that resulted in lowering the availability of carbohydrates for basal metabolism resulted in a shift to fat metabolism, which lowered CO<sub>2</sub> production. The consequence of the lower rate of CO<sub>2</sub> production was that breath-hold times were significantly increased, however this increased the risk of the breath-holding as the O<sub>2</sub> lever decreased below a recommended limit, thus increasing the risk of loss of consciousness, particularly if combined with pre-breath-hold hyperventilation. Practically, this increase risk of loss of consciousness was reversed by feeding the subjects with a high glucose drink. The effects of reduced carbohydrate availability were also shown by Dr. Lindholm to be magnified if there was also a reduction in muscle glycogen by prolonged exercise pre-breath-old, which significantly increased the risk of loss of consciousness during/after a breath-hold.

The third contribution to breath-hold diving safety resulted from Dr. Lindhom's study that experienced breath-hold divers have a greater resistance to the build-up of CO<sub>2</sub>, thus can breath-hold significantly longer, particularly when combined with other techniques to extend breath-hold time. Experienced breath hold subjects depended to a large extent on their hypoxic drive, whereas the less experienced subjects in the present study aborted their breath holds at or slightly before the hypoxia started to enhance the hypercapnic respiratory drive. The lower CO<sub>2</sub> sensitivity when combined with hyperventilation and lung packing exposes breath-holder's to a greater risk of loss of consciousness as it would result in a greater decrease in O<sub>2</sub> saturation.

### ***Respiratory Muscle Performance:***

Studies in our laboratory have shown that there is reduced performance of respiratory muscles (fatigue) at the end of an endurance run or swim conducted at 70-80% the subject's individual VO<sub>2 max</sub>. The demonstration of this was based on the drop in tidal volume toward the end of the run, which resulted in a significant increase in breathing frequency to meet the demands of the respiratory compensation for metabolic acidosis (lactate) mediated increased ventilation. These changes resulted in an increased VO<sub>2</sub>, presumably due to the increased work of breathing.

Studies in our laboratory also demonstrated that in air (running) voluntary isocapnic hypertension training of the respiratory muscles eliminated the drop in tidal volume and respiratory compensation for metabolic acidosis, and the run was stopped due to locomotor muscle fatigue. In addition it was demonstrated that voluntary isocapnic hypertension training and resistive respiratory muscle training performed for 30 min 5 times per week improved swim performance (33-66%) at the surface and at 4 fsw. The improved swim performance was greater with resistive respiratory muscle training.

Dr. Lindholm's contribution to this area was his study of the dose-response relationship between respiratory muscle training and the swim and pulmonary performance. This information was needed to optimize the training and minimize the time divers need to spend on training. Specifically, resistance respiratory muscle training was performed every other day, instead of five successive days per week, as is currently done. In addition the effects of maintenance training, twice per week, to maintain original performance gain were investigated. These studies revealed that training three days per week gave greater improvements in pulmonary and exercise performance than training 5 days per week. Furthermore, this study demonstrated that the performance gains after the four weeks of training 3 days/week were maintained by training 2 days/week in the maintenance phase. Also interesting was the observation that the 2 day/week training resulted in continuing improvement in maximal mouth pressure.

The continuation of the respiratory muscle training work led to testing the hypothesis that it would improve pulmonary and exercise performance at depth, where the work of breathing is significantly greater. At this stage of the work, Dr Ray took over the project and determined if resistance respiratory muscle training would prevent the reduction in pulmonary performance (respiratory muscle fatigue) and improve exercise performance of divers at depth of 55 and 120 fsw. These two studies clearly demonstrated that there was less respiratory muscle fatigue and that exercise performance was significantly improved at depth, in fact the magnitude of improvement was similar to that observed at the surface and 4 fsw.

Another conclusion of these studies was that respiratory muscle training enhanced the swimming performance of divers by reducing the respiratory work and increasing the efficiency of the respiratory muscles, thus allowing the muscles involved in swimming to not be compromised. The data to support these conclusions are based on the observations that after training, but not pre-training, the subject could sustain a tidal volume for the longer period of the swim. During the swim the total ventilation was less, as was breathing frequency, and the oxygen consumption was reduced, both observation consistent with increase muscle efficiency. In addition when the swim test was terminated at the same swim time, pre- and post-training, the maximal mouth pressure and respiratory muscle endurance were significantly greater post-training than pre.

Reduced CO<sub>2</sub> response, hypoventilation, increased end-tidal CO<sub>2</sub> and ensuing hypercapnia have been observed in escape tank instructors, trained underwater swimmers, and non-immersed divers in response to exercise while breathing air as well as O<sub>2</sub>. CO<sub>2</sub> retention has the potential to develop CO<sub>2</sub> narcosis and it also has a tendency to enhance central nervous system oxygen toxicity (CNS-OT) as well as nitrogen narcosis. Carbon dioxide retention alone may incapacitate a diver due to the narcotic effects of CO<sub>2</sub>, a phenomenon originally termed shallow water blackout as observed in oxygen breathing divers when the CO<sub>2</sub> scrubbers in their breathing gear functioned poorly. In divers that were CO<sub>2</sub> retainers it was demonstrated that they had a low ventilatory response to increased CO<sub>2</sub> (CO<sub>2</sub> sensitivity). While it could not be determined if the CO<sub>2</sub> sensitivity was cause of effect of CO<sub>2</sub> retention, it was demonstrated that respiratory muscle training increased CO<sub>2</sub> sensitivity, and led to a reduction of arterial CO<sub>2</sub> (CO<sub>2</sub> retention).

## **SIGNIFICANCE:**

Most scientists working in the systems integrated undersea medicine are in the latter stage of their career. Thus, fostering the next generation of scientists should be a high priority which was addressed in ONR BAA #04-13, and which was one of the aims of this project.. Two ONR Postdoctoral Fellow have been training and proceeded to independent careers in this field. In addition two Masters students and several undergraduate students have been exposed to the field.

Although Navy diving is very safe that are still problems for Navy divers. Two areas addressed during this grant period were the safety and performance of breath-holds and their ability to endure intense long-distance swimming.

As discussed previously deaths still occur in Navy missions and training that are associated with emergency or improper breath-holding techniques. The results of the present program emphasize that Navy divers should not become carbohydrate deficient, either through dietary restrictions or uncompensated exercise regimes, as this increase the risk of loss of consciousness during breath-holding. Breath-holding is a component of training and may be imposed by emergency situations experience by Navy divers.

Navy divers are routinely required to perform heavy and/or sustained exercise, especially when swimming long distances under water or against a current. Exertion while breathing on an underwater breathing apparatus (UBA) places a particularly severe demand on the respiratory muscles. Respiratory muscle fatigue might therefore become a mission limiting factor. The current studies demonstrated that respiratory muscle fatigue can be minimized by respiratory muscle training, and swimming performance increased, up to 66%. Strong support for the notion that these laboratory studies would apply to Navy personnel was also obtained in a study of very-shallow-water (VSW) divers at the Mine Counter Measures Detachment in San Diego. This project, which was supported by NAVSEA, showed that respiratory muscle training, either for improved endurance or strength increased the divers' swimming endurance substantially. The endurance swim time (time a weight could be supported) was recorded before and after RMT. After RMT, all divers (n=8) swimming endurance time had, on the average, increased 3 times, i.e. by 200%.

#### **PATENT INFORMATION:**

A patent disclosure has been submitted to the University at Buffalo's Technology Transfer office and is under review. Device and method for Respiratory Muscle Training. Lundgren ECG, Pendergast DR and Barth A.

#### **AWARD INFORMATION:**

1. Lundgren ECG: Distinguished Professor of the State University of New York
2. Pendergast DR: Nominated for Inventor of the Year Niagara Frontier Intellectual Property Association 2007

3. Pendergast DR: Albert R. Behnke Award for outstanding scientific contribution to advances in the undersea or hyperbaric biomedical field 2006
4. Pendergast DR: Honoree of the SUNY Research Foundation for Innovation, Creation & Discovery 2005
5. Pendergast DR: Exceptional Scholar Award for Sustained Achievement at University at Buffalo 2004
6. Pendergast DR: Nominated for Inventor of the Year Niagara Frontier Intellectual Property Association 2000

#### **REFEREED PUBLICATIONS:**

1. Pendergast DR, Lindholm P, Wylegala J, Warkander D, Lundgren CEG. Effects of respiratory muscle training on CO<sub>2</sub> sensitivity in SCUBA divers. 33(6): 447-455, 2006.
2. Lindholm P and Lundgren CEG. Alveolar gas composition before and after maximal breath-holds in competitive divers. , Undersea and Hyperbaric Medicine. 2006; 33(6): 463-467.
3. Lindholm P, Conniff M, Gennser M, Pendergast DR, Lundgren C. Effects of fasting and carbohydrate consumption on voluntary resting apnea duration. European Journal of Applied Physiology. 100(4):417-25, 2007.
4. Lindholm P, Wylegala J, Pendergast DR, Lundgren CEG. Resistive Respiratory Muscle Training Improves and Maintains Endurance Swimming Performance in Divers. Undersea and Hyperbaric Medicine. 34:169-180,2007.
5. Ray A.D., Pendergast D.R. Lundgren C.E.G. Respiratory muscle training improves swimming endurance at depth. Undersea and Hyperbaric Medicine. (in Press 2008).

#### **BOOK CHAPTERS, SUBMISSIONS, ABSTRACTS AND OTHER PUBLICATIONS:**

1. Fahlman A, Wylegala J, Lindholm P, Pendergast DR, Lundgren CL. Twelve Sessions of Resistive Respiratory Muscle Training Improves Underwater Swimming Endurance, Undersea and Hyperbaric Medicine, June 2005
2. Lindholm P, Conniff M, Gennser M, Pendergast DR, Lundgren CE. Effects of fasting and carbohydrate replenishment on voluntary apnea duration in resting humans. 53<sup>rd</sup> Annual meeting Am Coll Sports Med, 5/31-6/3 06 Denver CO. Med Sci Sports Exerc 38(5), 2006.

3. Ray AD, Pendergast DR, Simpson A., Lundgren CEG. Respiratory muscle training enhances swimming and respiratory performance at depth. *Undersea and Hyperbaric Medicine* 34(4), 290, 2007.
4. Ray, A.D., Farkas, G.A., Pendergast, D.R. Co-activation of the Tongue Protrudor and Retractor Muscles is not Altered in Obese Zucker Rats. *American Thoracic Annual Meeting*, 2008.
5. Ray A.D., Pendergast D.R., Simpson A. Lundgren C.E.G. Respiratory Muscle Training Against a Resistance Improves Respiratory and Underwater Swimming Performance. *American College of Sports Medicine Annual Meeting* 2008.
6. Ray AD, Simpson ALE, Pendergast DR and Lundgren CEG. Respiratory and exercise performance are improved and the work of breathing reduced at depth following respiratory muscle training. *Undersea and Hyperbaric Medical Society Annual Meeting*, 2008.
7. Simpson ALE, Ray AD, Pendergast DR, Lundgren CEG. Energy cost of breathing at depth. *Undersea and Hyperbaric Medical Society Annual Meeting*, 2008.
8. Ray AD, Simpson ALE, Pendergast DR, and Lundgren CEG. Respiratory and Exercise Performance are Improved and the Work of Breathing is Reduced at Depth Following Respiratory Muscle Training *Undersea and Hyperbaric Medical Society Annual Meeting*, 2008.
9. Ray AD, Simpson ALE, Pendergast DR and Lundgren CEG. Respiratory and exercise performance are improved and the work of breathing reduced at depth following respiratory muscle training. *Undersea and Hyperbaric Medicine* (submitted 5/08)